Remote MRI Sensing of pH and Cell Viability using Immunoprotective Microcapsules Crosslinked with Polycationic DIACEST Peptides

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Introduction Cell transplantation is a potential treatment for various diseases such as type I diabetes, liver failure, and arterial or cardiovascular disorders. Encapsulation of cells inside semi-permeable and biocompatible microcapsules offers immunoprotection for both grafted cells and the host recipient. Cell viability and function are a critical component for successful therapy. We have recently developed a library of CEST peptides which produce contrast in a pH-responsive manner and also possess a broad range of physical characteristics such as charge and solubility[1]. Many of these compounds are cationic and could potentially be used to crosslink the two alginate layers in these microcapsules. Using this peptide library[1], we explored the use biodegradable DIACEST microcapsules for visualization by MTw and CEST imaging. The contrast produced by these capsules is pH dependent, therefore we hypothesize that this can be used to monitor biological activities[2] such as apoptosis and insulin release, which are accompanied by pH changes. This proof-of-concept was demonstrated *in vitro* by acquiring MR images of DIACEST microcapsules which encapsulated either functional or apoptotic pancreatic beta cells.

Methods Pancreatic cells were first encapsulated in alginate beads gelled by 20 mM Ba^{2+} and then cross-linked by addition of a 0.05% polycationic DIACEST peptide[1] solution and further cross-linked by addition of a second layer of alginate. We experimented with multiple 12-residue polypeptides[1] such as lysine (K_{12}), lysine-glycine $(KG)_6$ and arginine-glycine $(RG)_6$, and compared the microcapsules produced to those generated using clinical-grade protamine (P). The MR images were acquired on a 9.4T Bruker Avance system equipped with a 15 mm sawtooth RF coil. We used a new two part CEST image acquisition scheme to compensate for the B_0 inhomogeneities present at high fields. A modified RARE (effective TE= 5.4 ms, RARE factor =16, slice thickness=0.3 mm, FOV=14x14 mm, matrix size=128x128, resolution= 0.11x011mm, and NA=2) sequence was used, with the addition of a magnetization transfer (MT) module. We acquired two series of images, one for measuring the water shift at each pixel, and a second to display the CEST contrast. The absolute water resonant frequency shift was measured using a modified Water Saturation Shift Reference (WASSR) method[3], TR=1.5 sec, t_{sat}= 500 ms, ω_1 =0.5 microT (21.3 Hz) and swept from -2ppm to 2 ppm (step= 0.1ppm). For the CEST-weighted images, saturation pulse ω_1 = 3.6 μT (150 Hz), saturation time=3sec, and TR=5.0 sec, with the offset swept from -6ppm to 6ppm (step=0.2-0.3ppm) around the water resonance (0ppm). Data processing was performed using custom-written scripts in Matlab. Z-spectra were calculated from the mean of ROI for each sample after B_0 correction. $MTR_{asym} = (S^{-\Delta\omega} - S^{+\Delta\omega})/ S^{-\Delta\omega}$ was computed at different offsets Δω (i.e. ±1.8 ppm and ±3.6 ppm) and used to display the CEST contrast.

Results&Discussion DIACEST microcapsules were clearly visible in both MTw and MTR_{asym} (CEST contrast) images (Fig. 1). Individual microcapsules could be detected in both MTR and MTR_{asym} images with the $(KG)_6$ capsules possessing the highest contrast in the MTR_{asym} images. In Figure 2A, we demonstrate that these capsules also display pH-dependent contrast. We varied the pH of solutions containing P microcapsules over a physiologically relevant range of 5.5-8.0, and found that the contrast depends strongly on pH with the MTR_{asym} ranging from 5-33%. (Fig. 2A) Next we were interested in determining whether this would be able to sense cell status *in vitro*. As shown in Fig. 2B, the MTR_{asym} for P microcapsules containing viable murine βTC6 insulinoma cells was ~15% higher than those containing 30-50% apoptotic cells (induced by treatment of 10μM of staurosporin) and ~10% higher than empty microcapsules. This demonstrates the potential of remote sensing of cell viability by CEST MRI. We were also interested in whether cells would remain functional after encapsulation in these new DIACEST microcapsules. As indicated by C-peptide secretion levels displayed in Fig. 3, human pancreatic beta islet cells encapsulated inside $(KG)_6$ microcapsules (Fig. 4B) were alive and functional for at least 27 days *in vitro,* with the C-peptide levels comparable to those using clinical-grade P capsules (Figure 4A).

Conclusion We have demonstrated the potential of DIACEST microcapsules for immunoprotection of engrafted pancreatic islet cells while providing a means for passive and pH-responsive imaging by MTw/CEST MRI. To our knowledge, these DIACEST microcapsules are the first immunoprotective capsules which can non-invasively monitor the viability and functionality of encapsulated cells as well as the distribution of cells and capsules in real-time without the use of metal-based contrast agents. Funding: NIH R01 EB007825 and K01 EB006394.

References 1. McMahon, M.T. *et al. Magn. Reson. Med*. **60**, 803-812 (2008).
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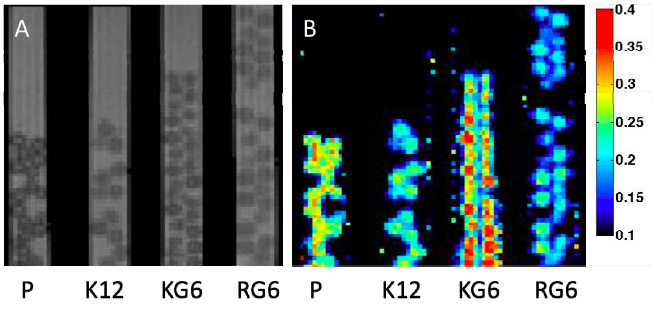

Figure 1. MTw (A) and CEST (B) images at 1.8 ppm of protamine (P), K_{12}, $(KG)_6$ and $(RG)_6$ microcapsules in 1-mm MR tubes.

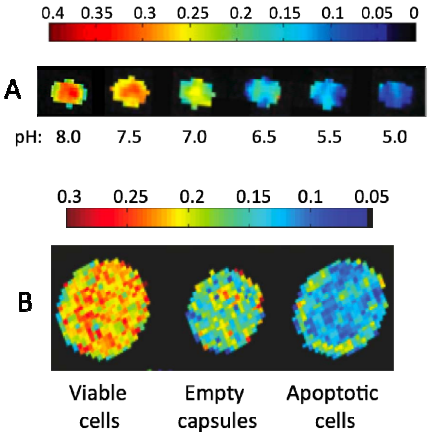

Figure 2. (A) CEST images of protamine microcapsules in solutions of various pH. (B) CEST images of protamine microcapsules containing viable and apoptotic murine βTC6 insulinoma cells and empty capsules in 1-mm MR tubes.

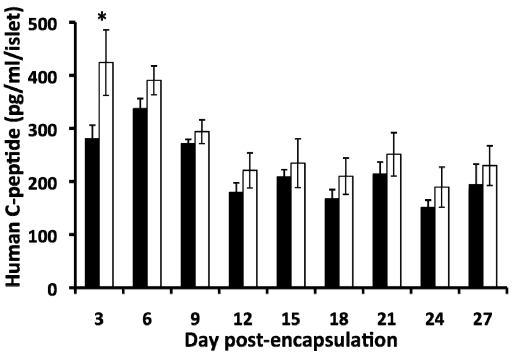

Figure 3. C-peptide secretion levels of human pancreactic islet cells encapsulated inside protamine (black) and $(KG)_6$ (white) capsules. *Time point when $(KG)_6$ level was statistically different than protamine ($P<0.05$).

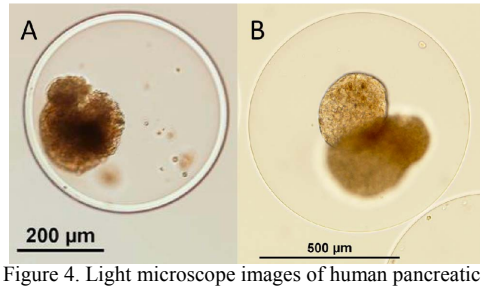

Figure 4. Light microscope images of human pancreatic islet cells inside a protamine (A) and $(KG)_6$ (B) microcapsule.